Name: **Neptun code:**

1. Open the hplcsimulatorapp to start the exercise and set up the starting parameters!
Column: Agilent Zorbax SB-C18
Particle size: 5 µm
Length: 150 mm
Diameter: 4.6 mm
Separable components: 3-phenilpropanol, Nitrotoluene, Phenol
Flow rate: 2 mL/min
Eluent: Acetonitrile:water 75:25%
Column temperature: 25°C

Chromatogram
5×8.5 cm

1.1.Illustrate the three separable components and put them in order by polaritiy!
Explain the sequence! (Proceed from the most polar to the least polar component!)

1.	**2.**	**3.**

1.2. Write the value of retention times and k primes of the components! Write the value of the void time as well!

t_{R1}= **k_1=**

t_{R2}= **k_2=**

t_{R3}= **k_3=**

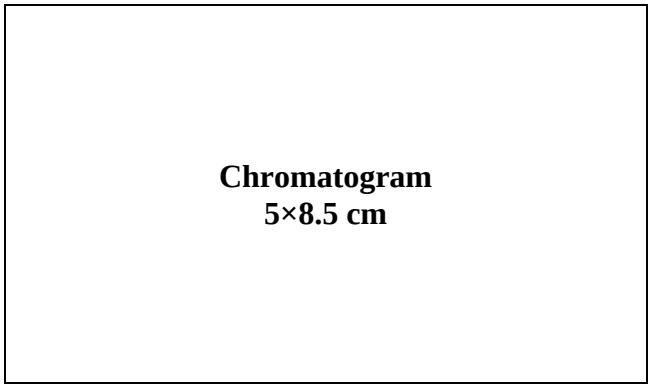
t_0=

1.3. Set up the eluent composition, in order that the three components separate from each other, but the time of the method does not take too much time!

The optimum of the eluent composition:

Explanation: (2-3 sentences/300 characters):

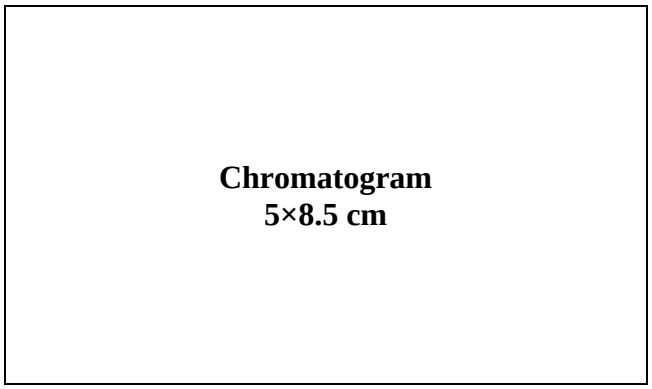
1.4.Keeping the eluent composition from the last question, change the acetonitrile to methanol! Write your experiences and give an explanation!



Explanation: (2-3 sentences/300 characters):

1.5. Set up an eluent composition where the three components are separated on the baseline and the time of the method is the shortest! Give the value of each retention time, k primes, theoretical plate and the resolution and selectivity between the first and second, and the second and third peeks!

t_{R1}=	k_1=
t_{R2}=	k_2=
t_{R3}=	k_3=
N=	perssure:
$R_{1,2}$=	$\alpha_{1,2}$=
$R_{2,3}$=	$\alpha_{2,3}$=



1.6.Compare the chromatograms which were made by acetonitrile and methanol! While working in a laboratory, where you have to detemine the concentrations of these three components in a large number of samples which eluent composition would you choose? Justify your answer!

2. For the answers of the next questions use the eluent composition which you determined in question 1.5! Answer the question in relation to the values (rention times, k primes, theoretical plate, resolution, selectivity) given in the 1.5. question!

2.1. Change the flow rate from 2 mL/min to 6 mL/min! Write down how the retention times, the theoretical plate and the back pressure change! Explain your observations!

Chromatogram
5×8.5 cm

t_{R1}= **N=**

t_{R2}=

t_{R3}= **pressure:**

Explanation: (2-3 sentences/300 characters):

2.2 Restore the starting parameters (except the eluent composition) change the length of the column from 150 mm to 50 mm! Write the retention times, the theoretical plate and the back pressure!

Chromatogram
5×8.5 cm

t_{R1}= **N=**

t_{R2}=

t_{R3}= **pressure:**

Explanation: (2-3 sentences/300 characters):

2.3. Restore the starting parameters (except the eluent composition) change the particle size of the column from 5 µm to 2 µm! Write down the retention times, the theoretical plate and the back pressure! Calculate the values of resolutions! Explain the change of values of theoretical palte and resolutions!

Chromatogram
5×8.5 cm

t_{R1}= **N=**

t_{R2}=

t_{R3}= **pressure:**

$R_{1,2}$=

$R_{2,3}$=

Explanation: (2-3 sentences/300 characters):

2.4. Restore the starting parameters (except the eluent composition) change the column temperature from 25 °C to 35 and 75 °C! Write the retention times, the theoretical plate and the back pressure! Calculate the values of resolutions! Explain the the change of values of the theoretical palte and resolutions!

Chromatogram **5×8.5 cm**	**Chromatogram** **5×8.5 cm**
35 °C	**75 °C**
t_{R1}=	**t_{R1}=**
t_{R2}=	**t_{R2}=**
t_{R3}=	**t_{R3}=**
$R_{1,2}$=	**$R_{1,2}$=**
$R_{2,3}$=	**$R_{2,3}$=**
N=	**N=**
pressure:	**pressure:**

Explanation: (2-3 sentences/300 characters):

2.5. What are the impotant things you have to consider, if you want to increase the temperature during the measurement? Write minimum two factors and justify your answers in 5-6 sentences!

2.6. Restore the starting parameters and set up the eluent composition to acetonitrile:water=10:90. Set up a gradient method with this starting eluent composition where the three components separate and the time of the method does not take too much!

Chromatogram 5×8.5 cm	**Chromatogram 5×8.5 cm**
acetonitrile:water 10:90	**by gradient**

Property of the gadient:

We have to use gradient method, when

3. You are working in a laboratory as chromatographer and are given the task that you have to make an HPLC method to separate the components of a given sample. First of all, which parameters must you change to separate the components of the sample? Which parameters are provide larger effect for the separation and which parameters are used for fine-tunings? Justify your answers using your knowledge and your explanations in the previous questions! Consider that certain parameters can be easily changed, but others only with great limitations (for example: changing the column)! (minimum 1500 characters)